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A SIMPLE METHOD FOR DETERMINING VARIATIONS IN THE HYDROGEN-ION CONCENTRATION OF THE BLOOD *

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Human blood, as it exists in the body, is faintly alkaline in reaction: that is, it has a hydrogen-ion concentration only slightly less than that of pure water, and this degree of alkalinity tends to be maintained even when considerable quantities of acid are produced within the body, or are introduced from without.

To a relative increase in the acid content of the body, the term "acidosis" is applied. Acidosis, conceivably, may be brought about in other ways than those just mentioned—for example, by decreased excretion of acid or by loss of bases from the body. The condition has been recognized in a variety of ways, such as increase in the ammonia coefficient of the urine, decrease of carbon dioxid tension in the alveolar air, the finding of abnormal acids in the blood and urine, by increased alkali tolerance and by diminished titratable alkalinity of the blood serum, by changes in the hemoglobin dissociation curve and by actual determination of the hydrogen-ion concentration of the blood. Production of ammonia, lowering of carbon dioxid tension in the alveolar air and in the blood, and excretion of acids are all part of the compensatory mechanism of the body, and it is only when this mechanism becomes overtaxed that appreciable changes in the hydrogen-ion concentration of the blood occur. The blood itself, owing chiefly to the "buffer" action of the carbonates of the plasma and phosphates of the corpuscles, can take up considerable amounts of acid or alkali without much change in its reaction. It is principally due to the work of L. J. Henderson[1] and his collaborators that we have a clear conception of the mode of action of these "buffers." An appreciable change in the hydrogen ion concentration of the blood indicates a failure of the protective mechanism and the presence of a significant acidosis. Herein lies the peculiar value of a determination of the hydrogen-ion concentration of the blood.

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* From the Pediatric and Medical Clinics, Johns Hopkins Hospital.

1. For a full discussion, see Henderson, L. J.: Ergebn. d. Physiol., 1909, viii, 298.

A brief word of explanation may be given for those unaccustomed to the physicochemical methods of expressing the reaction of a solution. A solution is acid when it contains an excess of hydrogen over hydroxyl ions, neutral when hydrogen and hydroxyl ions are in equal numbers, and alkaline when hydroxyl ions predominate. An acid of "normal" strength contains, in one liter, a gram of hydrogen capable of forming hydrogen ions,[2] and its strength may be expressed as 1 N. Diluting such a solution ten times, we would have 1/10 N or a solution containing 1/10 gram of actual or potential hydrogen ions to the liter. Continuing the process of dilution until 1/10,000,000 normal acid is obtained, we would have in such a solution 1/10,000,000 gram of hydrogen ions. Pure water, however, dissociates to form hydrogen and hydroxyl ions, and at 20 C. contains approximately 1/10,000,000 gram of hydrogen ions to the liter and an equivalent amount of hydroxyl ions (that is, 17 gm.). That is to say, pure water, our standard of neutrality, is 1/10,000,000 N acid and also 1/10,000,000 N alkaline. To avoid writing large figures it is customary to use the logarithmic notation and to express 1/10,000,000 N as 10^{-7}N or, more conveniently, as suggested by Sörensen,[3] to drop the 10 and minus sign and say[4] pH7. If we have less than 1/10,000,000 gram of hydrogen ions to the liter, the solution is less acid than water, that is, it is alkaline — so, pH8 means actually 1/1,000,000 N alkali. The higher the exponent the more alkaline, or what is saying the same thing, the less acid is the solution.

To sum up:

pH1 = N/10 acid.
.
.
.
pH6 = N/1,000,000 acid.
pH7 = *NEUTRALITY*.
pH8 = N/1,000,000 alkali.
.
.
.
pH14 = N/10 alkali.

The reaction of the blood serum varies approximately between pH7 and pH8, the neutral point, pH7 being reached only in severe uncompensated acidosis, and a reaction of pH8 being attained perhaps only after administration of alkalies.

The measurement of the pH of the blood as compared with that of inorganic solutions presents many difficulties. The standard gas chain electrometric method has been applied to the blood by a number of investigators. A full review of the literature up to 1910 is to be found in an article by Botazzi.[5] More recent determinations have been made

2. For the sake of simplicity we will consider here only a "strong" acid and assume it to be completely ionized in dilute solution.

3. Sörensen: Ergebn. d. Physiol., 1912, xii, 401.

4. CH7 and $[H^+] = 7$ are synonymous expressions. Intermediate values, as, for example, between pH7 and pH8, are commonly expressed in one of two ways, as 0.25×10^{-7} or pH = 7.6. The latter method is used in this paper. The conversion of one expression into the other is simple. For example, log. 0.25 = — 0.602, then $0.25 \times 10^{-7} = 10^{-0.602} \times 10^{-7.0} = 10^{-7.602}$ or pH = 7.602.

5. Botazzi: Der Harn sowie die übrigen Ausscheidungen und Körperflussigkeiten, edited by C. Neuberg, Berlin, 1911.

by Hasselbalch and Lundsgaard,[6] Peters,[7] Peabody[8] and Milroy.[9] The results have not been altogether uniform, and the accuracy of the method as applied to blood is questionable on account of the necessary reduction of the hemoglobin, and the influence of the protein substances present.

The fact that the gas chain method of determining the pH of the blood requires a delicate and expensive piece of physicochemical apparatus as well as considerable technical training has been standing in the way of clinical investigations of acidosis directly from the side of the blood, and has led to the very valuable but indirect methods of approach, such as those utilized in the studies of Sellards,[10] Palmer and Henderson[11] and Adler and Blake.[12]

PRINCIPLE OF THE METHOD

Heretofore the indicator method has not proved of great value in the studies of hydrogen-ion concentration of the blood, although the reaction of inorganic solutions may be determined quite accurately by this means.[13] Different indicators show their color changes at varying degrees of hydrogen-ion concentration; for example, the color of methyl orange changes from pink to yellow as the pH of its solution changes from 3 to 5. At intermediate points, various colors may be obtained and a certain color indicates a definite pH. Similarly, phenolphthalein changes from colorless to pink between pH8 and pH10 and can be used for the measurement of H-ion concentrations between these two points. In carrying out the indicator method, it is necessary to have a series of standard solutions of known pH and an indicator exhibiting easily distinguishable color changes at hydrogen-ion concentrations approximating that of the solution under consideration. It is then simply necessary to add equal amounts of indicator to the standard solutions and to the solution being tested and to determine which of the colors in the standard solutions most closely matches that of the unknown solution.

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6. Hasselbalch and Lundsgaard: Biochem. Ztschr., 1912, xxxviii, 77. Lundsgaard: Ibid., 1912, xli, 247.
7. Peters: Physiol. Soc. Prac., January, 1914.
8. Peabody, F. W.: Studies on Acidosis and Dyspnea in Renal and Cardiac Disease, THE ARCHIVES INT. MED., 1914, xiv, 236.
9. Milroy: Quart. Jour. Exper. Physiol., 1914, viii, 141.
10. Sellards: Johns Hopkins Hosp. Bull., 1912, xxiii, 289; ibid., 1914, xxv, 101.
11. Palmer, W. W., and Henderson, L. J.: Clinical Studies on Acid Base Equilibrium and the Nature of Acidosis, THE ARCHIVES INT. MED., 1913, xii, 153.
12. Adler, H. M., and Blake, Gerald: The Retention of Alkali by the Kidney with Special Reference to Acidosis, THE ARCHIVES INT. MED., 1911, vii, 479.
13. For a full description of the application of indicators to this purpose, see Sörensen: Ergebn. d. Physiol., 1912, xii, 393.

This method has been successfully used on the urine by Henderson[14] and by Walpole.[15] As proteins interfere with the colors of many indicators, and as both blood and serum possess color, it has been impossible to apply the method directly to the blood.[16]

It seemed probable that the indicator method might be utilized for blood, provided coloring matters and proteins could be excluded by means of dialysis.[17] If blood is dropped into collodion sacs and dialyzed for five minutes, the dialysate is free from proteins and coloring matter, but contains salts, and is well adapted to the use of indicators.

Since phenolsulphonephthalein exhibits definite variations in quality of color, with very minute differences in hydrogen-ion concentration between pH6.4 and 8.4, it was adopted as the indicator in this method.

PREPARATION OF STANDARD COLORS

Standard phosphate mixtures are prepared according to Sörensen's[18] directions as follows:

1/15 mol. acid or primary potassium phosphate. 9.078 grams of the pure recrystallized salt (KH_2PO_4) is dissolved in freshly distilled water and made up to 1 liter.

1/15 mol. alkaline or secondary sodium phosphate. The pure recrystallized salt ($Na_2HPO_4.12H_2O$) is exposed to the air for from ten days to two weeks, protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $Na_2HPO_4.2H_2O$ is obtained; 11.876 grams of this is dissolved in freshly distilled water and made up to 1 liter. The solution should give a deep rose red color with phenolphthalein. If only a faint pink color is obtained, the salt is not sufficiently pure.

The solutions are mixed in the proportions indicated below to obtain the desired pH:

pH	6.4	6.6	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	8.0	8.2	8.4
Primary Potas. Phos., c.c.	73	63	51	37	32	27	23	19	15.8	13.2	11.0	8.8	5.6	3.2	2.0
Secondary Sodium Phos., c.c.	27	37	49	63	68	73	77	81	84.2	86.8	89.0	91.2	94.4	96.8	98.0

Three c.c. of each of the solutions are placed in suitable small test tubes (100 × 10 mm., inside measurement). Five drops of an aqueous

14. Henderson: Biochem. Ztschr., 1910, xxiv, 40.

15. Walpole: Biochem. Jour., 1910, v, 207.

16. Sörensen (Biochem. Ztschr., 1909, xxii, 238) has applied the indicator method to the filtrate obtained after precipitating blood or serum by boiling with three volumes of dilute hydrochloric acid. Adler (Am. Jour. Physiol., 1907, xix, 1) has tested the reaction of the serum with paper dyed pink with rosolic acid. The objections to these methods are obvious.

17. Preliminary experiments showed that it was impossible, by the indicator method, to obtain concordant results on dilutions of serum.

18. Sörensen: Biochem. Ztschr., 1909, xxii, 352.

0.01 per cent. solution of phenolsulphonephthalein are added to each tube. The tops are sealed off. The series of colors, representing different concentrations of hydrogen ions, constitutes the standards for comparison of color in carrying out the determination.[19]

PREPARATION OF SACS

One ounce of celloidin (Anthony's negative cotton)[20] is dissolved in 500 c.c. of a mixture of equal quantities of ether and ethyl alcohol. The solid swells up and dissolves with occasional gentle shakings, in forty-eight hours. As a small amount of brown sediment separates out at first, the solution should stand for at last three or four days, after which the clear supernatant solution is ready for use.[21] A small test tube (120 by 9 mm. inside measurement) is filled with this mixture, inverted, and half the contents poured out. The tube is then righted, and the collodion allowed to fill the lower half again. A second time it is inverted and rotated on its vertical axis, the collodion being drained off. Care must be taken to rotate the tube, in order to secure a uniform thickness throughout. The tube is clamped in the inverted position and allowed to stand for ten minutes, until the odor of ether finally disappears. It is filled five or six times with cold water, or it is allowed to soak five minutes in cold water. A knife blade is run around the upper rim, so as to loosen the sac from the rim of the test tube, and a few cubic centimeters of water are run down between the sac and the glass of the tube. By gentle pulling the tube is extracted, after which it is preserved by complete immersion in water.[22]

THE SALT SOLUTION USED IN THE METHOD

The blood or serum is dialyzed against an 0.8 per cent sodium chlorid solution.

Before applying the test, it is necessary to ascertain that the solution is free from acids other than carbonic. To determine this, a few cubic centimeters of the salt solution are placed in a Jena test tube and one or two drops of the indicator added, whereupon a yellow color appears. On boiling, carbon dioxid is expelled, and the solution loses its lemon color and takes on a slightly brownish tint. In the absence of this change, other acids are present, and the salt solution is therefore not suitable.[23] If, on the other hand, on adding the indicator, pink at once appears, the solution is alkaline and hence cannot be used.

TECHNIC OF METHOD

The technic can be carried out on either serum, plasma, whole or defibrinated blood. The work must be done in a room free from fumes of acids or ammonia.

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19. The colors may fade slightly in a month's time, but may still be used for comparison if less indicator is added to the "unknown" solution, as the color quality remains the same.

20. Obtained from the Ansco Co., Binghamton, N. Y. This contains 30 per cent. of water and must be rinsed with absolute alcohol before being dissolved for use.

21. Better sacs are obtained from solutions that have been allowed to "age" in well-stoppered bottles for from two to three weeks.

22. Sacs that have dried became brittle and impervious.

23. It is advisable to keep the salt solution in a Jena glass flask and to protect it from acids in the air by means of a soda lime tube.

One to three c.c. of clear serum[24] or of blood is run, by means of a blunt pointed pipet, into a dialyzing sac which has been washed inside and outside with salt solution and which has been tested for leaks by filling with the salt solution.[25] The sac is lowered into a small test tube (100 by 10 mm., inside measurements) containing 3 c.c. of the salt solution, until the fluid on the outside of the sac is as high as on the inside. From five to ten minutes are allowed for dialysis.[26] The collodion sac is removed and 5 drops of the indicator are thoroughly mixed with the dialysate. The tube is then compared with the series of standards until the corresponding color is found, which indicates the hydrogen-ion concentration present in the dialysate.

These tests have been carried out with 3 c.c. of blood or serum. The same results are obtained with 1 c.c. of blood or serum on the inside of the sac, and with this amount it is immaterial whether there is 1 or 3 c.c. of salt solution on the outside.

COMPARISON OF TUBES WITH STANDARDS

For this, a good light (natural or artificial) and a white background are requisites. Readings must be made immediately. The tube matching[27] most closely is selected and also the tubes on either side of it. These are critically inspected against a white background. Changing the order of the tubes often makes differences more apparent.[28]

CONTROLS OF THE METHOD

Repeated duplicate determinations on the same samples of blood and of serum have convinced us that the limits of error are very slight: for example, the serum from a case of mild acidosis (using quantities of serum varying from 1 to 3 c.c. and dialyzing for from five to fifteen minutes) gave the following series of readings: 7.55, 7.55, 7.55, 7.55, 7.6, 7.55, 7.55, 7.55, 7.55, 7.55. The oxalated whole blood from the same case gave the following readings under similar conditions: 7.25, 7.25, 7.25, 7.25, 7.2, 7.25, 7.25, 7.3, 7.25, 7.25, 7.25, 7.25, 7.25, 7.25.

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24. Hemolysis tends to increase the acidity and must be avoided.

25. A sac may be used more than once for serum, provided it is thoroughly washed.

26. The alkalinity of the dialysate increases rapidly during the first five minutes. There is no appreciable change during the next ten minutes. Proteins may make their appearance in the dialysate in from ten to twenty minutes.

27. It must be borne in mind that one is here matching the quality of color and not intensity as in ordinary colorimetry.

28. A color falling between two of the standards may be read, by interpolation, to another decimal place. No effort was ever made to read closer than 0.05. Duplicate determinations should be made in all cases when sufficient material is available.

In order to test out the effect of the variations in the sacs used, a number of determinations were made on the same sample of serum with the following results: ordinary thin sac, 7.7; thick sac, 7.7; opaque, irregular sac, 7.7; ordinary thin sac, 7.65; very thick sac, 7.7. A series of six normal serums were run through, 3 c.c. and 1 c.c. portions being used for dialysis. In every instance identical readings were obtained

APPLICATION OF THE METHOD

Having thus assured ourselves of the accuracy of the method, a series of bloods from normal and pathologic cases were studied, with the following results:

1. *Normal individuals;* twenty-five cases. (*a*) Serum; Twenty four of the twenty-five cases read between 7.6 and 7.8. In one instance, 7.9 is recorded:

pH	Cases
7.6	4
7.65	1
7.7	5
7.75	5
7.8	3
7.9	1

(*b*) Whole blood (oxalated[2]); nineteen determinations. These all read between 7.4 and 7.6:

pH	Cases
7.4	3
7.45	2
7.5	4
7.55	5
7.6	5

The slightly greater acidity of whole blood as compared with serum has been recognized by almost all investigators in this field, and appears to be due to the fact that hemoglobin, and especially oxyhemoglobin, behaves as a weak acid

(*c*) Defibrinated blood. Early in the course of the work, defibrinated blood was run in parallel series with serum and oxalated whole blood. It was found that in general, the results tended to correspond to those obtained with the whole blood, but the range of variation was rather wide: from 7.4 to 7.8. No additional information was obtained, and the defibrination merely served to complicate the procedure. Hence it was decided to abandon the use of defibrinated blood in the subsequent course of the investigation

2. *Miscellaneous medical cases.* In order to determine whether the hydrogen-ion concentration of the blood varies from the normal in disease, a rather large variety of conditions was studied. Sixty-three

[illegible]

determinations were made in 52 cases, comprising the following conditions: nephritis (acute and chronic), 12 cases; diabetes mellitus, 8 cases; myocardial insufficiency, 5 cases; syphilis, 4 cases; chronic arthritis, 3 cases; pernicious anemia, 2 cases; neuroses, 3 cases, and 1 case each of pyelitis, empyema, carcinoma of neck, hemorrhoids, typhoid fever, pneumonia, meningococcus meningitis, tuberculous meningitis, cholelithiasis (jaundice), pulmonary edema, cerebral hemorrhage (coma), tuberculous pleurisy, foot and mouth disease, angioneurotic edema and brain tumor.

The results were as follows:

(*a*) Serum; sixty-three determinations. Sixty of the sixty-three determinations read between 7.6 and 7.8:

pH	Cases
7.6	14
7.65	4
7.7	19
7.75	1
7.8	22

The three exceptions not coming within these limits were:

(1) An instance of traumatic neurosis with a reading of 7.9. (2) A febrile typhoid with a reading of 8.0. (3) A case of chronic nephritis with hematemesis from an acute gastric ulcer, whose serum after the transfusion of 850 c.c. of blood, by the syringe method, gave a reading of 7.95.

(*b*) Whole blood (oxalated); thirty-three determinations, of which thirty-one read between 7.4 and 7.6.

pH	Cases
7.4	1
7.45	2
7.5	10
7.55	8
7.6	10

The two exceptions were instances of pernicious anemia. The first case, immediately following transfusion, gave a reading of 7.7 on the whole blood, this being identical with that on the serum. It is of interest to note that before transfusion the readings on the serum and whole blood were also the same, that is, 7.6. The second case gave a reading of 7.65 in the whole blood, this again being identical with that on the serum.

3. *Acidosis.* From the preceding paragraphs it is evident that the hydrogen-ion concentration of the whole blood or serum, normally and in a great variety of disease conditions, is not subject to great variations. A small series of cases with clinical or laboratory evidence of acidosis (or both) has been studied, the results appearing in Table 1. Fifteen determinations were made in eight cases.

TABLE 1.—Acidosis (Eight Cases, Fifteen Determinations)

Case No.	Date	H-Ion Concentration: Serum	H-Ion Concentration: Whole Blood (Oxal.)	Diagnosis and Remarks
1	12 26 14	....	7.1	Congenital cystic kidneys; adherent pericardium; myocardial insufficiency; hydrothorax; ascites; uremia; phthalein, trace in two hours; CO_2 tension of alveolar air, 19.5 mm. Hg.
	1 2 15	7.4	.	Dull, drowsy; phthalein, still on. [illegible] in two hours.
2	12 3 14	7.2		Acute and chronic nephritis; hypertension; uremia; phthalein, trace in two hours; Sellards' test, complete decolorization on evaporation
3	1 13 15	7.55		Eclampsia; mild toxemia.
4	1 25 15	7.5	7.2	Eclampsia; severe toxemia. NH_3 nitrogen in urine, 18 per cent.
	1 29 15	7.8	7.5	Thirty-six hours after labor, alkali therapy, doing well
5	1 18 15	7.2		Sarcoma of kidney and antrum, acidosis; sudden onset of acidosis with "air hunger"; Sellards' test, complete decolorization on evaporation; acetone and diacetic acid in urine
	1 19 15	7.45		After large doses of alkali; better; Sellards' test, faint pink color before complete evaporation
	1 28 15	7.8		More alkali; no dyspnea.
	2 18 15	7.3		Respirations again deep and [illegible], acetone in urine
6	1 22 15	7.5		Recurrent vomiting; no food retained for three days; acetone and diacetic acid in urine
7	1 22 15	7.4		Alimentary intoxication
	1 22 15	7.55		After alkali therapy; death next
8	1 8 15	7.4		Acute and chronic nephritis; myocardial insufficiency (slight), dyspnea, dyspnea; phthalein 55 per cent. in two hours, acetone in urine
	1 20 15	7.6	7.5	Much improved, CO_2 tension of alveolar air, 40.1 mm. Hg

[illegible] to Dr. J. Whitridge Williams for permission to study [illegible] in his [illegible]

CHANGES IN THE HYDROGEN-ION CONCENTRATION OF THE BLOOD IN EXPERIMENTAL CONDITIONS

An effort has been made to see if similar changes in the pH of the blood and serum can be demonstrated in experimental acidosis in dogs. Animals were injected intravenously with dilute hydrochloric acid. Protocols of two experiments are given in Tables 2 and 3.

TABLE 2.—PROTOCOL OF EXPERIMENT 1.—INTRAVENOUS INJECTION OF N/3 HYDROCHLORIC ACID INTO A DOG* (FEMALE, WEIGHT 7.3 KG.)

Time p. m.	Amt. of HCl Injected c.c.	H-Ion Concentration: Serum	H-Ion Concentration: Whole Blood (Oxal.)	H-Ion Concentration: Blood Drawn Directly Into Sac	Remarks
3.43		7.75	7.6	7.55	
3.44-3.49	50				
4.00	..	7.65	7.4	7.45	Breathing normally
4.14	..	7.7	7.45		
4.17-4.23	40				
4.26	..	7.6	7.5	7.4	No essential change in condition.
4.50-4.56	30	....	....	....	Toward end of injection, respirations slow and deep, 14 per minute.
5.00		7.65	7.55	7.5	Marked "air hunger", respirations, 11 per minute; pulse, 31 per ¼; regular.
5.03					Respirations still slow but shallower; animal in fairly good condition.
5.27-5.30	40	....	....	....	At 5.26 respirations 11 per minute.
5.45	..	7.45	7.45	7.4	Restless; struggling
5.[illegible]					Off table; no "air hunger"; lying down in no apparent discomfort; recovered.

[illegible] in part into [illegible], in part into leg vein. Blood for examination was [illegible] leg vein on opposite side. HCl made up in 0.8 per cent salt [illegible]

GENERAL DISCUSSION

In order to determine the actual hydrogen ion concentration of the blood as it exists in the body, it is necessary that measurements be made at body temperature and under the carbon dioxid tension existing in the vessels as shown by analysis of the alveolar air. Most of the published results do not fulfil these two requirements and therefore do not give the reaction, as Lundsgaard[31] expresses it, *strictissimo sensu*

[illegible]

In a study of acidosis, however, these considerations are of academic rather than of clinical importance, since we are concerned with variations in the pH rather than with its actual value.

The more recent electrometric measurements have been carried out at the carbon dioxid tension existing in the alveolar air and in most cases at room temperature (18 C.). So long as the measurements are made at a constant tension of carbon dioxid and at a constant temperature, the results are of value for comparative purposes.

TABLE 3.—Protocol of Experiment 2.—Intravenous Injection of N/2 Hydrochloric Acid Into a Dog (Female, Weight 7 Kg.)

Time	Amt. of HCl Injected	H-Ion Concentration		Remarks
		Serum	Whole Blood (Oxal.)	
3.21	....	7.7	7.55	Serum light yellow; clear
3.28	Injection begun			
3.36	....	....	....	Beginning to breathe deeply and slowly; heart's action very forceful; regular
3.50	90 c.c. have been injected.	6.9	6.9	"Air hunger" marked; respirations, 28 per minute. Heart's action forceful; regular; serum shows moderate amount of hemolysis
3.56	Injection ended, 105 c.c. have been injected			
4.00	....	6.9	6.9	In extremis; gasping for breath, heart still beating strongly, serum shows marked hemolysis
4.09	....	....	....	Ceased breathing; heart stopped beating; artificial respiration and intravenous injection of 5 per cent. solution of sodium bicarbonate unavailing.

In the method described in this paper, blood is exposed to the air so that the carbon dioxid tension is low, but apparently, fairly constant. The objection might be made that the pH would depend on the amount of carbon dioxid which has escaped from the blood or serum. The following experiments indicate, however, that the pH is not dependent, within reasonable limits, on the time elapsing between the taking of the blood and the determination of the pH, provided the

tube containing blood is kept stoppered and on ice, as will be seen from the following:

	Serum	Oxalated Blood
Immediately	7.75	7.55
After 2½ hours	7.75	7.55
After 19 hours	7.75	7.55
After 24 hours	7.75	7.55

If, however, blood is drawn directly into a sac immersed in a tube of salt solution and the reading compared with that from the blood drawn and determined in the usual manner, slight variations are encountered (see Experiment 1).

By thoroughly shaking the blood or serum in the air, its alkalinity is increased. Therefore, shaking should, in general, be avoided, unless it is desired to determine the pH at a zero CO_2 tension. Such studies have been carried on by Dr. D. W. Wilson.

The influence of the temperature on the readings has been determined, and the results appear in the accompanying tabulation.

TABLE 4.—Variations in Hydrogen Ion Concentration Due to Changes in Temperature

	A		B	
Temperature	Serum	Whole Blood (Oxal.)	Serum	Whole Blood (Oxal.)
20 C.	7.75	7.55	7.75	7.45
30 C.	7.85	7.5	7.8	7.4
37 C.	7.9	7.65	7.9	7.45

This effect of temperature has been recognized by previous workers. It is evident that in order to obtain comparable results, temperature control is necessary. Our measurements have been made between 20 and 24 C.

The dialysate of the blood or serum is slightly more acid than the original material, if one can judge from experiments carried out with dilute sodium bicarbonate solutions containing carbon dioxid, and phosphates of approximately the pH and concentration of blood. That the variation is a constant one under the conditions of the method is shown by the close agreement of duplicate determinations, which has been referred to previously in this paper.

By a coincidence, the results obtained from the dialysate from the whole blood and from serum correspond very closely to those obtained directly by the electrical method when the latter measurement is made at 18 C. and at a carbon dioxid tension of 40 mm. Hg.

This agreement in results is probably due to the antagonistic character of the two main sources of error involved in the method of dialysis, namely, loss of carbon dioxid tending to give higher, that is, more alkaline readings, and disproportionate dialysis of acid and basic constituents yielding lower, that is, more acid readings. For clinical pur-

poses, particularly the study of acidosis, the method of dialysis is quite as applicable as the electrometric method, and yields results in general comparable with those of that method. It has the advantages of simplicity and rapidity.

CONCLUSIONS

1. The indicator method of determining H-ion concentration is made applicable to blood and serum by utilization of dialysis through a collodion membrane, which excludes the disturbing influences of color and of proteins. The method is simple, accurate, rapid and well adapted for clinical use.

2. The technic consists of dialyzing 3 c.c. of blood or serum at room temperature against 3 c.c. of 0.8 per cent. salt solution for five minutes, adding an indicator and comparing with colored standard phosphate mixtures of known H-ion concentration.

3. Phenolsulphonephthalein is employed as the indicator in this method. It is found to exhibit easily distinguishable variations in quality of color, with minute differences in H-ion concentration between the limits pH6.4 and pH8.4.

4. Oxalated blood from normal individuals gives a dialysate with a pH varying from 7.4 to 7.6, while that of serum ranges from 7.6 to 7.8.

5. Variations from these figures toward the acid side were encountered only in conditions which clinically, and from the standpoint of the laboratory findings, evidenced an acidosis.

6. In a small series of clinical acidoses, the serums varied from 7.55 to 7.2 and the oxalated blood from 7.3 to 7.1. In experimental acidosis in dogs, a pH of 6.9 has been encountered in both serum and blood just before death.

REPORT OF CASES

CASE I.—H. S. (Medical No. 33,441), a white man, aged 43. Diagnosis: congenital cystic kidneys, adherent pericardium, myocardial insufficiency, edema of lungs, hydrothorax, and uremia. Admitted Dec. 12, 1914.

The patient had good general health until ten years ago. He had muscular rheumatism at eight. Eighteen years ago he had an attack of "acute Bright's disease," which confined him to bed for two weeks. Ten years ago, a lump was discovered in the left flank. Exploratory operation revealed congenital cystic kidneys and a few of the cysts in the right kidney were punctured. Five years later the left kidney also became palpable.

The present illness began five months before the patient's admission, with attacks of nocturnal dyspnea, which gradually became worse. Three weeks ago swelling of the legs appeared.

Examination showed an undernourished man, dyspneic and orthopneic. Signs of fluid were present in both pleural sacs, with bubbling râles over the lower lobes. There was marked cardiac enlargement and systolic retraction of interspaces lateral to apex. Broadbent's sign was present, also a protodiastolic gallop. There was a faint systolic blow at the apex, which was transmitted to the axilla. The pulmonic second sound was accentuated. The abdomen was

distended with fluid. A large mass covered with bosses was palpable in each flank. The liver was enlarged and tender. Edema of legs was noted.

Repeated phenolsulphonephthalein tests showed the excretion of only a trace in two hours.

The CO_2 tension of alveolar air was: December 16, 23.3 mm. Hg; December 18, 19.1 mm. Hg.

The hydrogen-ion concentration of blood was: December 26, whole blood, 7.1; January 2, serum, 7.4.

The nonprotein nitrogen of the blood was 118 mg. per hundred cubic centimeters. The blood pressure ranged from 155 to 135 mm. Hg systolic, 110 to 75, diastolic.

The urine was pale and clear with a specific gravity of 1.008 to 1.012. A trace of albumin and hyaline casts were observed. Acetone was present on several occasions. The patient was discharged Jan. 11, 1915, somewhat improved.

Case 2.—C. O. P. (Medical No. 33,385), a white man aged 43. Diagnosis: arteriosclerosis, acute and chronic nephritis, hypertension and uremia. Admitted Dec. 1, 1914. The patient had syphilis twenty years ago and was treated by intermittent courses of mercury for four years. He had uncomplicated typhoid fever twenty-three years ago and from 1908 to 1912 drank heavily.

The present illness began two years ago with severe headaches, usually coming on in the early morning hours and becoming gradually more severe. Polyuria and nycturia soon followed. Three months ago he had an attack of sudden loss of consciousness with a generalized convulsion lasting five or six minutes, and followed by profuse vomiting. He was in bed for four days. There was no paralysis. Eight weeks before his admission blood was discovered in the urine. He was put to bed and has remained there since. Two months ago his eyesight began to fail; he is now unable to read.

Examination showed a fairly nourished man with pasty complexion and decidedly anemic appearance. The heart was moderately enlarged. There was a presystolic gallop, with numerous extrasystoles. The second aortic sound was loud and ringing. The radials and brachials were diffusely thickened; the temporals tortuous and sclerosed. Advanced albuminuric retinitis was noted, with fresh hemorrhage in both eyes.

The blood count showed red blood cells, 3,520,000; white blood cells, 11,400; hemoglobin (Sahli) 65 per cent.

By the phenolsulphonephthalein test, excretion in two hours on numerous occasions ranged from a trace to 16 per cent. December 3, the patient was vomiting and belching at frequent intervals and was very drowsy.

Sellard's test on the blood serum showed complete decolorization on evaporation, no color on addition of water.

The hydrogen-ion concentration of the serum was 7.2. During the patient's stay in the hospital the blood pressure ranged from 208 to 174 systolic; 136 to 108 diastolic.

The urine in quantity was 1,025 to 5,025 c.c. in twenty-four hours; its specific gravity, 1,005 to 1,009. Smoky at first, it became clear with the polyuria, then bloody again. From 2 to 5 grams of albumin per liter were present. Hyaline, granular and blood casts were observed. The patient refused to remain for treatment and was discharged unimproved, Dec. 22, 1914.

Case 3.—F. W. (Obstetrical Service), a colored girl, aged 18, was admitted Jan. 8, 1915. Diagnosis: pre-eclamptic toxemia; postpartum eclampsia. The girl was nine months pregnant. On the day before admission, albumin and casts were found in the urine, and the patient was advised to enter the hospital. During the course of the next few days she complained of epigastric discomfort and frontal headaches of increasing severity. The systolic blood pressure was 190 mm. Hg. On January 12, slight edema of the legs appeared.

On January 13, at 7:55 a. m., spontaneous labor occurred. Ten minutes after the birth of the child the patient had a typical eclamptic convulsion of moderate

severity lasting two minutes. The blood pressure (systolic) at this time was 150 mm. Hg. There were two more convulsions that day. Following bleeding, purging and sweating, a normal convalescence ensued.

The hydrogen-ion concentration of the serum obtained at venesection was 7.55.

The nonprotein nitrogen in the blood was 42 mg. per 100 c.c.

January 24, the phenolsulphonephthalein test showed 55 per cent. excretion in two hours.

The ammonia nitrogen in the urine:

January 13 = 5 per cent.
January 14 = 9 per cent.
January 16 = 7 per cent.

Case 4.—M. K. (Obstetrical Service), a white woman, aged 19, was admitted Jan. 25, 1915. Diagnosis: eclampsia; spontaneous labor.

The patient was a strong, well-nourished primipara, nine months pregnant. The pregnancy had been uneventful until the morning of January 25, when, at 4 a. m., she began to have severe headache, nausea and vomiting. Shortly afterward she had one convulsion. When seen in the out-patient service she was conscious but drowsy. Blood pressure (systolic) was 150 mm. Hg; the pulse 80 per minute. The urine showed 8 grams of albumin per liter.

She was admitted to the ward at 7 p. m., unconscious, exceedingly restless, and having typical, moderately severe eclamptic convulsions at intervals of three-quarters of an hour. The blood pressure (systolic) was 180 mm. Hg. There was no dyspnea and very slight edema of lower legs. She had seven convulsions in all, the last occurring at 5 a. m. on the morning of January 26. Blood for hydrogen-ion determination, obtained after the third convulsion, gave a reading of 7.5 on the serum; 7.3 on the whole blood.

Following venesection, purgation and sweating, and the administration of 40 grams of sodium bicarbonate by stomach-tube, the convulsions finally ceased and consciousness returned on the morning of January 26. On January 28 a dead child was born spontaneously. The puerperium was uneventful.

The ammonia nitrogen of the urine during the three days preceding delivery ranged from 18 to 10 per cent.

January 29, the hydrogen-ion concentration of blood was for the serum, 7.8; for the whole blood, 7.5.

Case 5.—L. C. (Harriet Lane Home No. 6,538), a white boy, aged 3, was admitted Jan. 13, 1915. Diagnosis: sarcoma of the kidney and antrum; acidosis.

The boy had been a normal, healthy child up to three months before admission, when, following a trauma to the face, a small, red swelling appeared on the left cheek, subsequently pushing through to the roof of the mouth. Though painless, this swelling had gradually grown larger.

Examination showed a well-developed, well-nourished child. There was a firm mass in the left cheek, apparently arising in the antrum, and pushing down the left side of the hard palate in the mouth. There was a nodular, firm mass, the size of a man's fist, in the right umbilical region.

January 16: Breathing was rapid and deep.

January 17: There was marked dyspnea; the respirations were 40 per minute

January 18: Respirations unusually deep, with actual "air hunger." The urine contained acetone and diacetic acid.

Sellards' test showed colorless on complete evaporation. The hydrogen-ion concentration of the serum was 7.2. One hundred and seventy-five c.c. of a 5 per cent. solution of sodium bicarbonate was given by the Murphy method. This was followed by 175 c.c. of a 4 per cent. solution of sodium bicarbonate intravenously. After the first 50 c.c., respirations became noticeably less labored, and shortly after the completion of the injection the child was quite comfortable. Small doses of bicarbonate were continued by mouth.

January 19: Sellards' test showed a faint pink color before complete evaporation.

The hydrogen-ion concentration of the serum was 7.45. The child was breathing normally, though mentally rather dull.

During the succeeding weeks several radium treatments were administered and it was believed that the abdominal mass had grown somewhat smaller.

On February 18 the respirations became suddenly deep and noisy. The child seemed drowsy. The urine showed a trace of acetone, but no diacetic acid. The patient was given alkali by mouth.

The hydrogen-ion concentration of the serum was 7.3.

On February 25 the patient was discharged. Breathing again was normal though the general condition was failing.

CASE 6.—A. McK. (Harriet Lane Home), a white boy, aged 6, was admitted Jan. 21, 1915. Diagnosis: recurrent vomiting.

The boy had been a normal child except that he had always had a "weak stomach." Three days before admission, following an indiscretion in diet, he complained of abdominal pain. Next day he began to vomit and since then had not retained any food except a little orange juice. He vomited without any apparent effort, and after vomiting seemed quite well.

Examination showed a sparely nourished child, apparently quite normal in all respects. The urine contained acetone and diacetic acid. The hydrogen-ion concentration of the blood serum was 7.5. Following a well-regulated dietary regimen, he rapidly improved and went home.

CASE 7.—V. D. (Harriet Lane Home No. 6,628), a white girl, aged 2 months, was admitted Jan. 22, 1915; died Jan. 23, 1915. Diagnosis: alimentary intoxication.

The patient was a full-term, apparently healthy, child. Sixteen days before admission she began to vomit after every feeding and had frequent green stools. The vomiting and diarrhea persisted up to the time she was brought to the hospital.

Examination showed a small, moderately well-nourished baby, conscious and crying fretfully. Slight cyanosis was present. The respiratory rate was increased, the respirations being full and deep. White blood cells, 22,170. The stool, large, green and watery, contained mucus and food residue.

Sellards' test showed no color on evaporation or on addition of distilled water after evaporation.

The hydrogen-ion concentration of the serum was 7.4.

Intensive alkali therapy was given, both intravenously and subcutaneously. The respirations improved a little, becoming shallower. There was one slight convulsion and constant convulsive twitchings of the face and extremities.

The hydrogen concentration of the serum after intravenous injections of sodium bicarbonate was 7.55.

The respirations gradually became shallower and more infrequent and the baby died at 4:55 a. m. on January 23. There was complete anuria during the stay in the hospital. Partial necropsy showed only enlargement of the mesenteric lymph nodes.

CASE 8.—M. F. (Medical Service), a white man, aged 20, was admitted Jan. 7, 1915. Diagnosis: acute and chronic nephritis, hypertension, and myocardial insufficiency.

The patient had frequent sore throats. Eighteen months ago he began to have morning headaches. For three months, dyspnea on exertion was noted, becoming progressively more marked. Six days before admission the feet and ankles began to swell, and the abdomen and face have since become edematous. Anorexia, dizzy spells and epistaxis have occurred during the past week.

Examination showed an overnourished young man, dyspneic, sallow and pale. Marked oral sepsis was present; the tonsils were large and ragged. Crepitant râles were heard at the left base. The heart was markedly enlarged.

There was a snapping aortic second sound. The liver was enlarged and tender. Edema of feet and ankles was present. There was a moderate secondary anemia.

The Wassermann test was negative.

The phenolsulphonephthalein test showed 55 per cent. of excretion in two hours. The hydrogen-ion concentration of the serum was 7.4. The nonprotein nitrogen of the blood was 36 mg. per 100 c.c.

January 20: The hydrogen-ion concentration of the serum was 7.6; whole blood, 7.5. The CO_2 tension of alveolar air was 40.1 mm. Hg.

The urine in quantity was 1,500 to 2,000 c.c. in twenty-four hours. The specific gravity was 1.012 to 1.013. There were 1.5 to 2.75 grams of albumin per liter. On admission, red blood cells and acetone were observed, which subsequently disappeared. Numerous granular casts were found.

The blood pressure ranged from 170 to 130 mm. Hg systolic; 110 to 65 diastolic. The patient is somewhat improved. He is still in the hospital (April, 1915).